

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number  
**WO 02/00875 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 15/10**,  
15/90, 15/64, 15/66

(71) Applicant (for all designated States except US): **PRO-  
TEMATION, INC.** [US/US]; 11661 NE Sunset Loop,  
Bainbridge Island, WA 98110 (US).

(21) International Application Number: PCT/US01/19770

(72) Inventor; and

(22) International Filing Date: 20 June 2001 (20.06.2001)

(75) Inventor/Applicant (for US only): **CLARK, Robin**  
[US/US]; 11661 NE Sunset Loop, Bainbridge Island, WA  
98110 (US).

(25) Filing Language: English

(26) Publication Language: English

(74) Agent: **CHEN, Shirley**; Wilson Sonsini Goodrich &  
Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050  
(US).

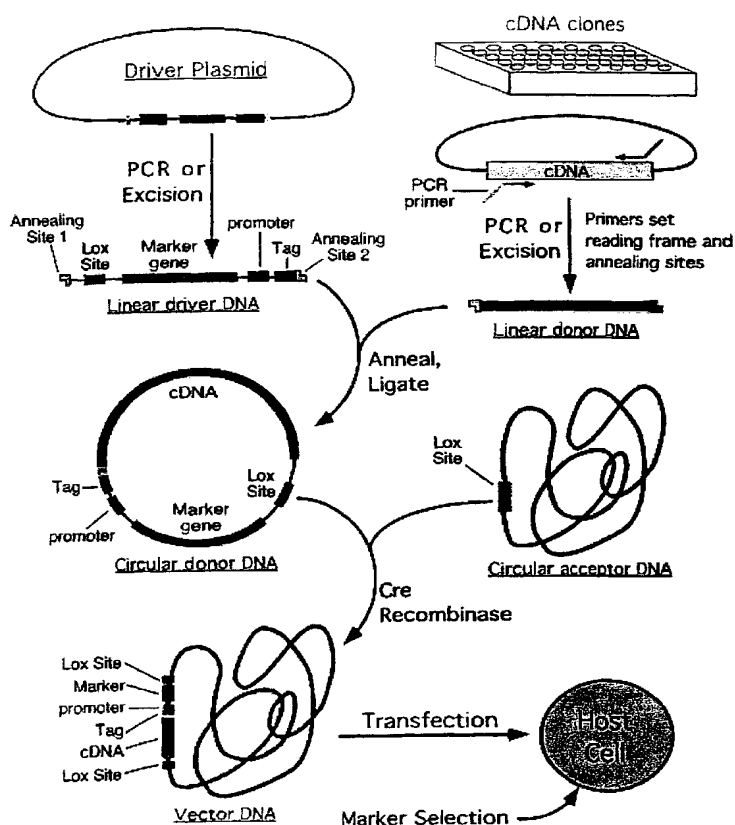
(30) Priority Data:  
09/606,323 28 June 2000 (28.06.2000) US

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,

(63) Related by continuation (CON) or continuation-in-part  
(CIP) to earlier application:  
US 09/606,323 (CON)  
Filed on 28 June 2000 (28.06.2000)

[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR GENERATING EXPRESSION VECTORS THROUGH SITE-SPECIFIC RECOMBINATION



(57) Abstract: Compositions, kits, and methods are provided for use in a recombinational cloning or subcloning methods for constructing expression vectors which comprise: ligating a library of double-stranded linear donor DNAs, where each member of the library includes a donor DNA sequence, with a double-stranded linear driver DNA which includes a promoter sequence and a donor recombination site to form a single circular donor DNA, the single circular donor DNA not including an origin of replication, where the donor DNA sequence is under the transcriptional control of the promoter; and contacting the circular donor DNA and a circular acceptor vector in the presence of a recombinase to form a single fused circular vector, the circular acceptor vector comprising an origin of replication and an acceptor recombination site capable of recombining with the circular donor DNA.



WO 02/00875 A3



SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

**(84) Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— with international search report

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

**(88) Date of publication of the international search report:**

2 May 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

In ational Application No

PCT/US 01/19770

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/10 C12N15/90 C12N15/64 C12N15/66

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 21977 A (LIFE TECHNOLOGIES INC) 6 May 1999 (1999-05-06) the whole document ---	
A	WO 96 40724 A (LIFE TECHNOLOGIES INC ;HARTLEY JAMES L (US); BRASCH MICHAEL A (US)) 19 December 1996 (1996-12-19) the whole document ---	
A	WO 00 12687 A (MC CONNELL GINA C ;MARCIL ROBERT (US); MILES DAVID J (US); TURNER) 9 March 2000 (2000-03-09) the whole document ---	
A	WO 00 29000 A (LIFE TECHNOLOGIES INC) 25 May 2000 (2000-05-25) the whole document --- -/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

26 February 2002

Date of mailing of the international search report

05/03/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/19770

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 47758 A (EINSTEIN COLL MED ;MASSACHUSETTS INST TECHNOLOGY (US)) 18 December 1997 (1997-12-18) the whole document ---	
P,A	WO 01 05961 A (CLONTECH LAB INC) 25 January 2001 (2001-01-25) the whole document ---	
P,A	WO 01 31039 A (INVITROGEN CORP) 3 May 2001 (2001-05-03) the whole document ---	
P,A	WO 00 52027 A (LIFE TECHNOLOGIES INC) 8 September 2000 (2000-09-08) the whole document ---	
P,A	WO 00 63410 A (EINSTEIN COLL MED ;LEBOULCH PHILIPPE (US); MASSACHUSETTS INST TECH) 26 October 2000 (2000-10-26) the whole document -----	

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/19770

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9921977	A	06-05-1999	AU 1199599 A CN 1280614 T EP 1025217 A1 JP 2002500861 T WO 9921977 A1 US 6277608 B1	17-05-1999 17-01-2001 09-08-2000 15-01-2002 06-05-1999 21-08-2001
WO 9640724	A	19-12-1996	AU 724922 B2 AU 6384196 A CA 2226463 A1 EP 0937098 A1 JP 11507236 T US 6171861 B1 WO 9640724 A1 US 6270969 B1 US 6143557 A US 5888732 A	05-10-2000 30-12-1996 19-12-1996 25-08-1999 29-06-1999 09-01-2001 19-12-1996 07-08-2001 07-11-2000 30-03-1999
WO 0012687	A	09-03-2000	AU 5584999 A EP 1114148 A1 WO 0012687 A1	21-03-2000 11-07-2001 09-03-2000
WO 0029000	A	25-05-2000	AU 1721600 A EP 1131078 A1 WO 0029000 A1	05-06-2000 12-09-2001 25-05-2000
WO 9747758	A	18-12-1997	US 5928914 A AU 733016 B2 AU 3306297 A CA 2258007 A1 EP 0914457 A1 JP 2000512150 T WO 9747758 A1	27-07-1999 03-05-2001 07-01-1998 18-12-1997 12-05-1999 19-09-2000 18-12-1997
WO 0105961	A	25-01-2001	AU 6346900 A WO 0105961 A1	05-02-2001 25-01-2001
WO 0131039	A	03-05-2001	AU 1437801 A WO 0131039 A1	08-05-2001 03-05-2001
WO 0052027	A	08-09-2000	AU 3614300 A EP 1173460 A1 WO 0052027 A1	21-09-2000 23-01-2002 08-09-2000
WO 0063410	A	26-10-2000	AU 4343300 A WO 0063410 A1	02-11-2000 26-10-2000